teach how to make or use the invention. The applicants have asserted from the data illustrated that the synthetic sequences constructed by the applicants exhibited a more uniform and greater toxicity to hornworms than the native gene, and the Examiner questions how that conclusion was reached in view of the data presented.

It is admitted that the data on Table I does how some variability. Such variability is inherent in genetic engineering of plants. Since foreign genes introduced into plant cells are inserted randomly, there is a certain inherent variability in the results achieved due, among other effects, to positional effects from where the gene is inserted into the plant genome. Thus the variability does not indicate at all that the synthetic sequences were not better than the native sequence, it simply was and is an inherent factor always present in the genetic engineering of plants. Regardless of this variability, the applicants assert that the data clearly shows that the synthetic sequences do, on average, achieve a higher level of efficacy in killing feeding insects than does the native sequence. The degree of variability is not due to the sequence used, but is due to the inherent nature of genetic engineering. It is for that reason that sufficient numbers of plants were generated so that averages could be ascertained. Note that of 52 plants which were genetically engineered containing the native sequence, only two rated "9" in the applicants' rating system, a rate which corresponds to about 4%. All of the synthetic sequences achieved results much better than that. In fact, the applicants' greatest

rate of success was achieved with the sequence which has the least modification although, as discussed further below, the applicants believe that the amino terminal modification is the most important in achieving the result described and claimed in the present application.

The Examiner also questions the distribution of clones into the 9 rating, since the Examiner argues it is not clear what criteria were used to establish the various categories. First, the application does make it quite clear how the categories of 5 and 9 were distinguished (discussion pages 16 to 18). It is also clear from the context of the application that the ratings of 6 to 8 were merely gradations in between those two extremes. Note also that the applicants' tests were conducted to be of great stringency. No neonatal insects were used. All the insects were "hardened" after birth by feeding on wild-type or non-transgenic plants. The plants were rated "9" only if the insect rapidly ceased feeding and died quite quickly. Even a rating of 5 indicated that the insect died, the rating indicating that it took several days of limited feeding before the insect died.

The applicants assert that the specification of the application is fully enabling, and that there is an adequate written description. The methodology used to construct the present synthetic gene sequence is fully disclosed. The codon table usage is included. A description on how to genetically engineer plants is included. There is nothing about the Examiner's criticisms which are relevant to the issue of adequate written description or enablement. The applicants have asserted

that there is an effect, and that it is beneficial. The Examiner seeks to quibble about the applicants' definitions of certain terms, but even if those terms had been left out of the specification, it would not be non-enabling. The applicants have described, since it is a fact, that the synthetic sequence has achieved a higher level of "killers" than did the plants transformed with the native sequence. That is true, and that is all that is necessary in this instance for enablement and an adequate written description. Accordingly, it is believed that this rejection to the specification is inappropriate, and a reconsideration of this rejection is respectfully requested.

The Examiner has rejected Claims 1 to 17 under Section 112, first paragraph, on the grounds that the disclosure is enabling only for claims limited to Manduca sexta. The applicants respectfully disagree for reasons stated below. Nevertheless, in order to isolate the issues remaining with the present patent application, the Examiner will note that Claims 15 and 17 had been amended to specifically recite plants which are toxic upon ingestion to Manduca sexta.

In general, the applicants feel, as will be discussed in further detail below, that a generic method for generally improving the performance of foreign genes in plants has been described and claimed in the present application. It is axiomatic that multiple examples are not required of the methodology is made clear and is fully enabling for other examples. The applicants assert that that is the case here, and

that the claims should not be limited as asserted by the Examiner.

The Examiner has also rejected Claims 1 to 17 under 35 U.S.C. Section 112, first paragraph, on the grounds that the claims should be limited to tobacco. Apparently the Examiner argues this based on a reading of the Fischhoff et al. paper, which allegedly shows that a full length B.t. sequence can be expressed in tomato. From that, the Examiner argues that that data is inconsistent with the data presented in the Barton et al. paper and Vaeck et al. paper, regarding expression of similar full length genes in tobacco. The applicants assert that this data is not in any way inconsistent, and that the fair reading of the papers is that the same effect is present in tomato as in tobacco.

First, it should be noted that the test use by Fischhoff et al. to assay for insect toxicity from the gene is an extremely sensitive one. Note that Fischhoff et al. are using neonatal insects (methods and materials page 812). Four larvae were applied to each leaf, and if any of the larvae were killed after four days the assay was positive. Fischhoff does apparently report (on page 810) that three, and only three, transgenic plants were recovered containing a full length chimeric B.t. gene. No characterization of the genes in those plants is made in the paper. It is further reported that only one of these three plants had detectable activity, with the level of mortality much lower than that which could be found in any plant containing a truncated gene. Thus, at best, Fischhoff presents equivocal

data that perhaps one, and only one, plant exhibited some very marginal toxicity to insects while expressing a full length gene. This is not inconsistent with, and is, in fact, very consistent with, the analysis of the Barton et al. paper which concluded that expression of intact genes is toxic to plant cells. It would appear that two out of three of Fischhoff's plants did not express, and therefore didn't experience lethality. Apparently the last plant expressed so poorly that it could marginally kill bugs without totally killing the plant. There is no recitation anywhere in Fischhoff of a full length B.t. being expressed in tomato at levels to provide effective control of insects, while still having a healthy plant. In fact, Fischhoff suggests just the opposite, by specifically suggesting that the use of a truncated sequence is necessary to get effective control of insects. In essence, there is nothing in Fischhoff in tomato which is in any way inconsistent with the results of Vaeck et al. or Barton et al. in tobacco, and there is no reason whatsoever to believe that the gene expression of these insecticidal proteins would vary as a function of plant species. Accordingly, the Examiner's rejection in this regard is poorly made, and should be reconsidered.

The Examiner has rejected Claims 1 to 17 under Section 112, first paragraph, on the grounds that the disclosure is only enabling for claims limited to dicots. While the applicants feel that this rejection is also poorly made, the applicants have limited the claims to dicot species in order to obviate this

grounds of rejection, for the purposes of gaining allowable subject matter.

Claims 1 to 17 are rejected under Section 112, first paragraph, on the grounds that the disclosure is enabling only for claims limited to claims which recite the upper sequence shown in Figure 2. The applicants assert that this rejection is very poorly made. The applicants have described a method of altering the genetic sequence of any gene which is sought to be expressed in plants. The method described by the applicants for increasing the efficacy of the expression of foreign genes in plants is logically applicable to any of a number of proteins sought to be expressed into plant species. Applicants have pointed out that the pattern of codon usage is only one of the mechanisms to which poor expression may be ascribed. Nevertheless, the applicants have proposed a solution to one of these mechanisms, and the fact that it is not a panacea to solve all problems in the expression of genes in plants does not render the contribution unimportant. For some genes sought to be expressed in plants, the present method may in fact be very unimportant. The applicants and their co-workers have been readily able to express certain other genes in plants without resorting to this technique. However, for some genes, particularly procaryotic ones which favor different patterns of codon utilization, the technique of the present application may be extremely important. There is no reason to believe that this improvement achieved by the applicants' method is limited to the particular protein described herein, which is only exemplarily of

procaryotic genes which are expressed poorly in plants. Accordingly it is believed that this rejection too narrowly views the present invention and is inappropriately made. Reconsideration of this rejection is respectfully requested.

The Examiner had rejected Claims 1 to 16 and 17 under 35 U.S.C. Section 103 over a combination of references including a paper by Murray et al. While the applicants filed a Declaration under Rule 131 to overcome the Murray et al. reference, the Examiner had refused to withdraw the rejection on the grounds that not all inventors signed the Declaration. While the applicants can find no authority for the proposition that all inventors are required to sign such a declaration, in the interests of getting past that point, the applicants are submitting herewith the Declaration of Kenneth A. Barton in which he joins with the declaration of the other inventor, Michael J. Miller, in repeating the same facts. Accordingly, there can be little doubt that this rejection has been overcome, since the Murray et al. paper is not a reference available against the claims of the present patent application.

The Examiner has also applied a new rejection under Section 103 asserting that the present invention is obvious over a combination of references. The references of importance with regard to this rejection are those by Hoekema et al., Grantham et al. and Vaeck et al. or Barton et al.

The Hoekema et al. paper describes how the expression of a yeast gene, which is very well expressed, can be hindered by the alteration of a pattern of codon usage in the yeast gene so as to

introduce in that gene codons which are less favored in other yeast genes. Note that it is specifically recited in Hoekema that the gene which Hoekema is investigating is one which has every single codon of the gene utilizing a codon which is preferred by yeast cells. Nowhere does Hoekema address the reverse side of the question. Hoekema does not describe the alteration of any poorly expressed genes in yeast for a preferred codon usage pattern to achieve better expression. Hoekema only demonstrates that well expressed genes can be degraded, and does not suggest or demonstrate that weakly expressed or poorly expressed foreign genes can be increased in a level of expression.

The Grantham et al. paper does contain a codon usage table for plants which bears some resemblance to the codon usage table derived by the applicants. Note that it is not identical, as, for example, for the amino acid glutamine, Grantham's table shows a different codon preferred from the table of the applicants' contained in Figure 1 herewith. Nevertheless, Grantham does show a codon usage table for plants.

The Vaeck et al. and Barton et al. papers both disclose that the amino terminal portion of the B.t. toxin gene when expressed in plant cells can be toxic to insects. Both papers teach that a shortened or truncated version of the native protoxin gene is necessary for effective toxicity to insects when expressed in plants. Both of those papers also report difficulty in gaining the expression of the gene in plants. Note that specifically it was recited that there were low levels of toxin mRNA obtained in

insect resistant transformant plants compared to the levels obtained using other inserted genes, even a gene immediately adjacent to the B.t. gene (Barton et al. page 1108). It is specifically for that reason that Ken Barton, principal author of the Barton et al. paper, sought to make the improvements described and claimed in the present patent application, of which that same Ken Barton is one of the inventors.

It is asserted by the applicants here that the combination of references cited by the Examiner does not indicate with the required degree of certainty that the present strategy would be effective to achieve increased efficacy in gene expression in plants. Hoekema does not show that poorly expressed genes can be made to express better. Hoekema, in fact, uses a particular gene which uniquely includes, at every codon within the gene, the preferred codon for the host, i.e. yeast. It is worthy of note that plant genes do not include such a faithful utilization of preferred codons. The Examiner can readily examine Figure 1, the table of codon usage determined by the applicants, and see that plant genes vary widely in their codon usage. All known plant genes do, in fact, include both representations of codons which are preferred in plants and also include representations of codons which are not preferred in plants. Since the mechanisms by which levels of expression are regulated are poorly understood, it was not at all certain prior to the work of the applicants here that it was not necessary for plant genes to have such a population of codons of less popularity in order to be properly regulated. If all native plant genes include less

popular codons within their sequence, how is it clear that a foreign gene inserted into plants which did not include such less popular codons would be properly expressed? It was not clear that this would work in advance. It was not clear that it would be advantageous. The Examiner can only reach this conclusion by applying hindsight to references which do not suggest the strategy employed by the applicants here.

No reference cited by the Examiner shows the construction of a synthetic gene sequence for use in plants. The only references cited by the Examiner which show genetically engineered plants, the references to Barton et al. and Vaeck et al., show the use of native sequences which have been truncated and joined to foreign promoters, but the coding sequences are otherwise identical to that obtained from the native species. Thus the creation of synthetic sequences coding for foreign genes is new to the present application, and has not previously been demonstrated to be an effective strategy for gaining good expression of foreign proteins in plants.

Specifically with regard to previous Claim 17 and the newly submitted Claim 18, the Examiner appears to have a misapprehension with regard to the claimed subject matter. On page 8 of the Office Action, the Examiner argues that the fact that the amino terminal portion only of the B.t. gene was altered by codon substitution by the applicants to achieve their beneficial result would not be unexpected, since only the amino terminal is used for toxicity. In describing the applicable B.t. genes, the amino terminal portion of the gene necessary for

toxicity is still fairly large, 600 amino acids being necessary for toxicity, see Barton et al., page 1105. Note that the alteration made by the applicants here, as described in the present patent application, was specifically recited in Claim 17 to be between 25 and 132 codons. In other words, of the amino terminal portion of the protoxin, that is the toxin gene, less than one-third of that was altered in order to achieve the beneficial increased expression of the gene. Nothing in the cited prior art suggested that by altering only a portion of the gene at the amino terminal end, which comprises less than one-third of the total coding sequence of the gene, an increase in expression as recited by the applicants here could be achieved. Note that the applicants' best results were obtained using the plasmid named pAMVBT2, which had the least alteration of its coding sequence, but which did have the all important change to preferred codon usage at the amino terminal portion of that gene. Thus the applicants have asserted that it is the amino portion of the genes which are most advantageously altered to a pattern of preferred codon usage. This phenomenon is not anywhere suggested in the references cited by the Examiner, in any form whatsoever.

Accordingly, the applicants assert that the combination rejection made by the Examiner is inappropriate. These references might be considered an invitation to experiment, but they do not demonstrate with a reasonable degree of certainty that the applicants work would result in a successful product.

Accordingly, it cannot support a rejection for obviousness, and this grounds of rejection should be reconsidered as well.

In the interest of candor, the applicants wish to bring two more things to the attention of the Examiner. Copies of two recently published European applications are presented herewith, European application number 359472 and European application number 385962. Both of these applications recite the modification of codon usage in plants to gain better expression of the B.t. gene in plants. The text of European application 359472 does not disclose any actual work in plants. The text of European application 385962 discloses results very similar to those achieved by the applicants here. In any event, it is believed that neither of these is prior art, unless and until, a corresponding application issues in the United States.

Also for the interest of candor, the applicants would like to bring to the attention of the Examiner one other incident. In October of 1988, the undersigned attorney for the applicants here was permitted to view the patent files of Agrigenetics at the offices of Lori Greenlee in Boulder, Colorado. At that time, the undersigned reviewed the contents to several patent application files. The undersigned was not permitted to make copies of any of the files examined. At that time, the undersigned saw a patent application which described the creation of synthetic genes for expressing B.t. in plants. The undersigned has no notes which evidence the serial number or inventorship of that application, but the undersigned now believes that that may be the application upon which priority was based for the above

European application 359472. That access by the undersigned is not deemed prior art to the inventors here for several reasons. First, it was not known to the inventors at that time. Secondly, the inventors had already created transgenic plants, and had demonstrated their toxicity against insects prior to that date. This may be confirmed by reference to the Declarations under Rule 131 submitted herewith, which have already established that the inventorship was prior to that date. This incident is mentioned only in the interest of complete candor with the Patent and Trademark Office.

Wherefore reconsideration of the merits of this patent application is respectfully requested. A separate petition for extension of time of two months is submitted herewith so that the response may be considered as timely filed.

Respectfully submitted,

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